Instruction Manual

Thermo Scientific KingFisher Pure Viral NA Kit

Revision 1.0

N14040 March 2013

Thermo SCIENTIFIC

Copyright

Disclaimer

Thermo Fisher Scientific reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without prior notice as part of continuous product development. Although this manual has been prepared with every precaution to ensure accuracy, Thermo Fisher Scientific assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information. This instruction manual supersedes all previous editions.

Products are for Research Use Only. Not for use in diagnostic procedures.

The Product will operate substantially in conformance with Thermo Fisher Scientific's published specifications.
THERMO FISHER SCIENTIFIC DISCLAIMS ALL OTHER WARRANTIES, WHETHER EXPRESSED OR IMPLIED, ORAL OR WRITTEN, WITH RESPECT TO THE PRODUCTS, INCLUDING WITHOUT LIMITATION ALL IMPLIED WARRANTIES OF PRODUCT QUALITY, CONDITION, DESCRIPTION, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE. THERMO FISHER SCIENTIFIC DOES NOT WARRANT THAT THE PRODUCTS ARE ERROR-FREE OR WILL ACCOMPLISH ANY PARTICULAR RESULT. THERMO FISHER SCIENTIFIC HEREBY EXPRESSLY DISCLAIMS ANY WARRANTY REGARDING RESULTS OBTAINED THROUGH THE USE OF THE PRODUCTS, INCLUDING WITHOUT LIMITATION ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS.

Exclusion of Liability

Thermo Fisher Scientific and its affiliates shall have no liability to an End User arising out of the use or inability to use the product, including, without limitation, for any loss of use or profits, business interruption or any consequential, incidental, special or other indirect damages of any kind, regardless of how caused and regardless of whether an action in contract, tort, strict product liability or otherwise.

Table of Contents

NOTE: For more details on storing the kit reagents, refer to "Storage Conditions" on page 6.

1

Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure Viral NA Kit

Item	KingFisher Pure Viral NA Kit	
Cat. No.	98070196	98070496
Package size	96 samples	384 samples
Proteinase K	5.2 ml	21 ml
Carrier RNA	1 vial	4 vials
Lysis Buffer	40 ml	155 ml
KingFisher Magnetic Beads	2 x 1.4 ml	10.6 ml
Wash Buffer 1 (conc.)*	55 ml	2 x 115 ml
Wash Buffer 2 (conc.)*	50 ml	3 x 50 ml
Nuclease-free water	30 ml	2 x 30 ml

* Addition of ethanol or isopropanol required.

The KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) is intended for the purification of viral nucleic acids from body fluids and swab samples, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head.

The user will need the KingFisher Flex or KingFisher Duo magnetic particle processor for conducting purification (Table 1-2). In addition, several common laboratory instruments and consumables are required to conduct an efficient purification. For more details, refer to Chapter 5: "Protocols and Pipetting Instructions". Suitable consumables for the KingFisher Duo and KingFisher Flex are listed in Table 1-3 and Table 1-4.

Storage Conditions

Upon arrival of the kit, store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C and the Carrier RNA in the original aluminum bag at -20°C. The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to three years from the manufacturing date.

Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade
- 100% isopropanol, molecular biology grade

Reagents required for specific sample pretreatments (refer to Chapter 5):

- 1 x PBS (pH 7.4)
- 0.9% NaCl solution
- TE buffer (pH 8.0)
- 0.5 M EDTA

Table 1-2. Thermo ™ KingFisher™ magnetic particle processors

Cat. No.	Product
5400100	KingFisher Duo magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head

Table 1-3. Thermo Scientific™ KingFisher™ Flex consumables

Cat. No.	Product	Package size
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs

Table 1-4. Thermo Scientific™ KingFisher™ Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003520	KingFisher Duo elution strip	40 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates, and elution strips for 96 samples)	1 box

2

Product Description

Introduction

The KingFisher Pure Viral NA Kit is designed for rapid automated purification of viral nucleic acids from various samples, such as plasma, serum, saliva, and urine, as well as from nasal, buccal and urogenital swabs, and blood samples using Thermo Scientific™ KingFisher™ instruments. The nucleic acids (NA) purified using the KingFisher Pure Viral NA Kit are of high quality and free of proteins, nucleases, and other contaminants or inhibitors. They are, therefore, suitable for direct use in many different downstream applications, such as qPCR (quantitative PCR), RT-qPCR (reverse transcription qPCR), and several other enzymatic reactions.

Intended Use

The KingFisher Pure Viral NA Kit is developed for purification of viral nucleic acids from various samples, such as plasma, serum, saliva, and urine, as well as from nasal, buccal and urogenital swabs, and blood samples using paramagnetic particles. The reagents and specific plastic consumables are designed for use with the KingFisher Flex and KingFisher Duo magnetic particle processors as part of an integrated system. The KingFisher Pure Viral NA Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the KingFisher instrument and the KingFisher Pure Viral NA Kit for any particular use, as the performance of the kits has not been validated for any specific organism or downstream application.

Principle and Procedure

The KingFisher Pure Viral NA Kit uses magnetic-particle technology for viral RNA and DNA purification. The Thermo Scientific™ KingFisher™ technology combines the speed and efficiency of nucleic acids purification with easy handling of magnetic particles. The purification process requires no phenol/chloroform extraction and needs very little hands-on time.

The first step of the protocol lyses the sample, after which the nucleic acids can bind to the surface of the KingFisher Magnetic Beads in the presence of isopropanol. The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. The following three effective wash steps dispose of proteins, cell debris, and any residual contaminants, while the nucleic acids bound to the KingFisher Magnetic Beads are transferred through the wash steps. Two different Wash Buffers are used, followed by an air drying step. High-quality nucleic acids are eluted into the nuclease-free water, and are ready for subsequent downstream processes.

Kit Specifications

The KingFisher Pure Viral NA Kit is designed for rapid automated preparation of highly pure viral nucleic acids from body fluids and swab samples using KingFisher magnetic particle processors. If a dispense step requiring the addition of isopropanol is excluded, the approximate processing time is 40 minutes for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo. The obtained nucleic acids can be used directly in various downstream applications.

Suitable sample materials for purification are cell-free body fluids, such as serum, plasma, saliva, and urine, as well as nasal, buccal and urogenital swabs, and blood samples. The sample type as well as the handling and storage of the sample affect the yield of the purified nucleic acids.

KingFisher Magnetic Particle Processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific™ KingFisher™ systems is based on the use of magnetic rods covered with a disposable, specially designed tip comb and plates or tubes. Use only Thermo Scientific™ KingFisher™ plastic consumables, as use of products from other manufacturers may cause unsuitable mixing or even instability in the KingFisher instrument.

The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents, including magnetic particles, are dispensed onto the plates according to the corresponding instructions. Dispensing can be carried out manually or partially automatically using automatic dispensers, for example, the Thermo Scientific™ Multidrop™ Combi and/or the Thermo Scientific™ Versette™. Thermo Scientific™ BindIt™ Software 3.2 can be used for running ready-made and optimized protocols for the Thermo Scientific™ KingFisher™ Pure Kits. It is also possible to transfer the developed protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments provide a rapid and automated solution for complicated and time-consuming purification processes, resulting in high-purity nucleic acids without risk of carryover or cross-contamination.

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables, and the easy-to-use BindIt Software 3.2. The KingFisher Pure Viral NA Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

KingFisher magnetic particle processors are intended for professional research use by trained personnel. Detailed information and user instructions for the KingFisher instruments can be found in their respective user manuals.

The BindIt Software 3.2 protocols optimized for the KingFisher Pure Viral NA Kit are available for the KingFisher Flex 96 and KingFisher Duo. For more information, go to www.thermoscientific.com/kingfisherinfo or contact your local authorized distributor.

Table 2-1. Overview of KingFisher Flex and KingFisher Duo magnetic particle processors

	KingFisher Flex		KingFisher Duo	
	96 formats	24 format	12 format	6 format
Processing volume	20–1000 µl*	200–5000 µl	30–1000 µl*	200–5000 µl
Capacity	Up to 96 samples per run (sample volume 200 µl)	Up to 24 samples per run (sample volume 1 ml)	Up to 12 samples per run (sample volume 200 µl)	Up to 6 samples per run (sample volume 1 ml)
Magnetic head	96 inter-changeable formats for PCR plate, KingFisher Flex 96 KF plate, Microtiter deep well 96 plate	24 format for KingFisher Flex 24 deep well plate	12-pin magnet head for Microtiter deep well 96 plate	6-pin magnet head for KingFisher Flex 24 deep well plate
Plates	KingFisher Flex 96 KF plate (20–200 µl), 96 well PCR plate, skirted (20–100 µl), Microtiter deep well 96 plate (50–1000 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)	Microtiter deep well 96 plate (50–1000 µl), KingFisher Duo elution strip (30–130 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)
Tip combs	KingFisher Flex 96 tip comb for PCR magnets, KingFisher Flex tip comb for KF magnets, KingFisher Flex 96 tip comb for deep well magnets	KingFisher Flex 24 tip comb for deep well magnets	KingFisher Duo 12-tip comb	KingFisher Duo 6-tip comb
Heating temperature	Heating block temperature from +5°C above ambient room temperature to +115°C		Heating block temperature from +10°C to +75°C, elution strip +4°C to +75°C at room temperature	

* See the details above on the Plates row.

3

Safety Information

The following components of the KingFisher Pure Viral NA Kit contain hazardous contents (Table 3-1).

Always wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions provided in the kit instruction manual. It is recommended that Good Laboratory Practice (GLP) is followed to guarantee reliable analyses.

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidium thiocyanate	Harmful by inhalation, in contact with skin and if swallowed. Liberates very toxic gas in contact with acids. Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Keep the container in a well-ventilated place. Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/safety data sheets.

Continued

Cont.

Reagent	Hazardous contents	Safety instructions
Proteinase K	Proteinase, Tritirachium album serine	May cause sensitization by inhalation. Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). This material and its container must be disposed of as hazardous waste.
Wash Buffer 1 (conc.)	Guanidinium chloride	Harmful if swallowed. Irritating to eyes and skin. Do not breathe gas/fumes/vapor/spray. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste.

4

Storage Conditions and Preparation of the Reagents

Storage Conditions

Upon arrival of the kit, store the KingFisher Magnetic Beads at +4°C and the Carrier RNA in the original aluminum bag at -20°C. The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to three years from the manufacturing date.

Preparation of the Carrier RNA

To prepare the Carrier RNA solution, add 600 µl of nuclease-free water to the vial of the Carrier RNA. Incubate the freshly reconstituted Carrier RNA for 5 min at room temperature, then mix thoroughly, and briefly centrifuge the vial. Use immediately or store in aliquots at -20 °C. Repeated freezing and thawing should be avoided.

Preparation of the Lysis Buffer

Before each NA purification, calculate the amount of Lysis Buffer needed. For purification of one sample, 200 µl of Lysis Buffer is required. Prepare a fresh mixture of Carrier RNA and Lysis Buffer by adding 25 µl of Carrier RNA to 1 ml of Lysis Buffer. Mix with a pipette or by vortexing.

Preparation of the Wash Buffers

Add 100% isopropanol to the bottles containing Wash Buffer 1 and 96–100% ethanol to the bottles containing Wash Buffer 2, as indicated below in Table 4-1 prior to the first use.

Table 4-1. Instructions for the preparation of Wash Buffer 1 and Wash Buffer 2. Add the indicated volume of 100% isopropanol or 96–100% ethanol to each bottle.

	96 samples (Cat. No. 98070196)		**384 samples (Cat. No. 98070496)**	
	Wash Buffer 1	Wash Buffer 2	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	55 ml	50 ml	115 ml	50 ml
Isopropanol (100%)	45 ml	–	95 ml	–
Ethanol (96–100%)	–	200 ml	–	200 ml
Total volume	100 ml	250 ml	210 ml	250 ml

After preparing each solution, mark the bottle to indicate that the step has been completed. The buffers can be stored at room temperature.

5

Protocols and Pipetting Instructions

Before beginning the protocol for purification of nucleic acids, carefully read through the *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) or the *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. No. N12420), and the *Thermo Scientific™ BindIt™ Software for KingFisher Instruments version 3.2 User Manual* (Cat. No. N07974).

BindIt Software protocols for the KingFisher Pure Viral NA Kit can be found in BindIt Software 3.2 and at www.thermoscientific.com/kingfisher.

Handling of KingFisher Magnetic Beads

A homogeneous distribution of the KingFisher Magnetic Beads in the container is essential before the beads are transferred to the wells in order to ensure a high consistency between the wells. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly. The paramagnetic beads have a tendency to sediment relatively quickly.

Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for nucleic acid purification from 200 µl of plasma or serum samples, using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Flex with 96 deep well plates.

When using the KingFisher Pure Viral NA Kit for the first time, prepare the Carrier RNA, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

When beginning a new purification procedure, prepare a fresh mixture of Carrier RNA and Lysis Buffer by adding 25 µl of Carrier RNA to 1 ml of Lysis Buffer. Mix with a pipette or by vortexing.

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

1. Take four empty Thermo Scientific™ Microtiter™ deep well 96 plates and two empty Thermo Scientific™ KingFisher™ Flex 96 KF plates.

2. Prepare the Sample plate (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the **Sample plate** and leave the plate at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Sample (plasma, serum or other)	200 µl
			Lysis Buffer including the Carrier RNA	200 µl
			Proteinase K	50 µl

3. Fill the other **plates** as follows.

Plate number	Plate type	Plate name	Content	Reagent volume per well
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	900 µl
3		Wash 2_1	Wash Buffer 2	800 µl
4		Wash 2_2	Wash Buffer 2	800 µl
5	KingFisher Flex 96 KF plate	Elution	Nuclease-free water	100 µl

4. Place a Thermo Scientific™ KingFisher™ Flex 96 tip comb for deep well magnets on a **Tip Plate** (i.e. an empty KingFisher Flex 96 KF plate).

5. Start the PURE_ViralNA_Flex96 protocol using the KingFisher Flex 96 and load the plates as instructed on the KingFisher Flex 96 instrument display.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 96 deep well head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the PURE_ViralNA_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE_ViralNA_Flex96 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and 100% isopropanol to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step at approximately 15 minutes after starting the protocol run, remove the plate from the instrument, and add the well suspended magnetic bead suspension and 100% isopropanol to the **Sample plate**.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	KingFisher Magnetic Beads* Isopropanol	25 µl 450 µl

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Sample plate back into the instrument and press **Start**. After the pause, the protocol will continue to completion.

8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. Store the purified nucleic acids accordingly. The purified nucleic acids are ready for use in downstream applications.

NOTE: The final RNA concentration in the nuclease-free water may increase if the purified NA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

Instructions for KingFisher Duo with 96 Deep Well Plates

These instructions are intended for nucleic acid purification from 200 µl of plasma or serum samples, using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Duo with 96 deep well plates.

When using the KingFisher Pure Viral NA Kit for the first time, prepare the Carrier RNA, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

When beginning a new purification procedure, prepare a fresh mixture of Carrier RNA and Lysis Buffer by adding 25 µl of Carrier RNA to 1 ml of Lysis Buffer. Mix with a pipette or by vortexing.

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

1. Take one empty Microtiter deep well 96 plate and one Thermo Scientific™ KingFisher™ Duo elution strip.

2. Prepare the **Viral NA plate** (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the rows. Note that row B is reserved for the tip comb and should be left *empty*. Note that rows C, D, and E are also left *empty*.

Plate name and type	Row	Row name	Content	Sample /reagent volume per well
Viral NA plate Microtiter deep well 96 plate	A	Sample	Sample (plasma, serum or other) Lysis Buffer including the Carrier RNA Proteinase K	200 µl 200 µl 50 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1	Wash Buffer 1	900 µl
	G	Wash 2_1	Wash Buffer 2	800 µl
	H	Wash 2_2	Wash Buffer 2	800 µl

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the nuclease-free water is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Nuclease-free water	100 µl

4. Place a Thermo Scientific™ KingFisher™ Duo 12-tip comb into **row B** on the **Viral NA plate**.

5. Start the PURE_ViralNA_Duo protocol using the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 12-pin magnet head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the PURE_ViralNA_Duo protocol. Insert the Viral NA plate and elution strip into the instrument as indicated on the KingFisher Duo display and press **OK**. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

When the KingFisher Duo is to be run as a standalone instrument, transfer the PURE_ViralNA_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and 100% isopropanol to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step at approximately 15 minutes after starting the protocol run, remove the plate from the instrument, and add the well suspended magnetic bead suspension and 100% isopropanol into **row A** on the **Viral NA plate**.

Row	Plate name	Content	Reagent volume
A	Sample	KingFisher Magnetic Beads*	25 µl
		Isopropanol	450 µl

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Viral NA plate back into the instrument and press **OK**. After the pause, the protocol will continue to completion.

8. When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and switch off the instrument. Store the purified nucleic acids accordingly. The purified nucleic acids are ready for use in downstream applications.

NOTE: The final RNA concentration in the nuclease-free water may increase if the purified NA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

Specific Instructions for Various Sample Materials

When using the KingFisher Pure Viral NA Kit for the first time, prepare the Carrier RNA, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

When beginning a new purification procedure, prepare a fresh mixture of Carrier RNA and Lysis Buffer by adding 25 µl of Carrier RNA to 1 ml of Lysis Buffer. Mix with a pipette or by vortexing.

Instructions for nucleic acid purification from 4.5 ml of urine

These instructions are intended for nucleic acid purification from 4.5 ml aliquots of urine samples, using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Flex or KingFisher Duo with 96 deep well plates.

1. Add 500 µl of 0.5 M EDTA to 4500 µl of urine. Urine samples may contain insoluble salt precipitates that can reduce nucleic acid yields, thus limiting the sample volume used for purification.

2. Centrifuge for 10 min at 800 × g. Discard the supernatant.

3. Resuspend the pellet in 200 µl of PBS or 0.9% NaCl and use as a sample in the purification.

4. Proceed with the purification following the corresponding instructions for the KingFisher Flex or KingFisher Duo with 96 deep well plates for nucleic acid purification from 200 µl of cell-free body fluid (see page 17 or page 20).

Instructions for nucleic acid purification from buccal and nasal swabs

These instructions are intended for nucleic acid purification from buccal and nasal swab samples, using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Flex or KingFisher Duo with 96 deep well plates.

1. To collect a swab sample, scrape the swab 5–6 times against the inside of a cheek or nose.

2. Swirl the swab for 2–3 min in 200 µl of PBS or 0.9% NaCl and use the solution as a sample in the purification.

3. Proceed with the purification following the corresponding instructions for the KingFisher Flex or KingFisher Duo with 96 deep well plates for nucleic acid purification from 200 µl of cell-free body fluid (see page 17 or page 20).

Instructions for nucleic acid purification from saliva

These instructions are intended for nucleic acid purification from saliva samples, using the KingFisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the KingFisher Flex or KingFisher Duo with 96 deep well plates.

1. Centrifuge the saliva sample for 5 min at 3,000 × g. Discard the supernatant.

2. Resuspend the pellet in 200 µl of PBS or TE buffer and use as a sample in the purification.

3. Proceed with the purification following the corresponding instructions for the KingFisher Flex or KingFisher Duo with 96 deep well plates for nucleic acid purification from 200 µl of cell-free body fluid (see page 17 or page 20).

Quantification and Determination of the Purity of Nucleic Acids

The amount of viral nucleic acids in cell-free body fluids is usually very small and the yields of purified nucleic acids are low. In addition, the Carrier RNA is copurified and affects the analyses of the yield with a spectrophotometer. To determine the yield of viral nucleic acids, it is recommended to use amplification techniques.

6

General Information

Reagent Specificity and Volumes

A reagent must not be used with any kit other than that for which it is intended. It is strongly recommended that the volume of reagents in each well or tube is kept within the limits specified in the *KingFisher Flex User Manual* or *KingFisher Duo User Manual* to avoid spillover and to maximize efficiency of performance.

Handling of Magnetic Beads

The KingFisher Magnetic Beads should be mixed thoroughly before use to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes. The amount of beads in the wells or tubes affects the yield of the purified nucleic acids.

Binding, Wash, and Elution Steps

The binding between the nucleic acids and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt. The binding will remain throughout the wash steps until the elution where the nucleic acids are released.

The volume of the nuclease-free water in the elution can be modified depending on user requirements concerning the purified nucleic acid concentration. The final nucleic acid concentration in the nuclease-free water may increase if the purified nucleic acids are eluted into a smaller than recommended volume of the buffer, but this can slightly reduce the overall nucleic acid yield. The modifications of the elution step must be done in BindIt Software 3.2 and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

Table 6-1. Available elution volumes of the KingFisher Flex and KingFisher Duo

KingFisher instrument	Elution volumes
KingFisher Flex with 96 deep well head, elution in a KingFisher Flex 96 KF plate	50–150 µl
KingFisher Flex with 96 deep well head, elution in a Microtiter deep well 96 plate	50–1000 µl
KingFisher Flex with 24 deep well head	200–5000 µl
KingFisher Duo with 12-pin magnet head, elution in an elution strip	30–130 µl
KingFisher Duo with 12-pin magnet head, elution in a Microtiter deep well 96 plate	50–1000 µl

To maximize the yield of purified nucleic acids, avoid the lowest permitted volumes of elution in the KingFisher instruments. The nuclease-free water in the elution should cover the KingFisher Magnetic Beads completely, and any possible magnetic-bead pellet(s) should be completely resuspended. In addition, the volume of the nuclease-free water should be adequate for efficient mixing of the beads in order to obtain a maximal release of the purified nucleic acids from the beads.

Decontamination and Disinfection of Sample Material

You should decontaminate the sample material and the reagents and plastics that have been in contact with the sample material in order to minimize the risk of contamination. Use a decontaminant, such as Virkon™, paying due attention to the manufacturer's instructions. You should also take care of the appropriate treatment and/or disposal of waste.

A

Troubleshooting

Problem	Possible cause and actions
Low NA yield	Make sure that the Carrier RNA was added to the Lysis Solution. Make sure that isopropanol was added to Wash Buffer 1 and ethanol to Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on page 16. Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency. Efficient lysis of the samples increases the total NA yield. Prolonged storage of the sample material may reduce the NA yield. There should be an adequate volume of water to completely cover the KingFisher Magnetic Beads during the elution step. Use only Thermo Scientific plates, strips, and tip combs with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified NA.
Low purity	Insufficient washing causes impurities in the eluted NA.

Continued

Cont.

Problem	Possible cause and actions
Magnetic particles remaining in the sample or elution well	Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the particles unless the viscose samples are diluted before the beginning of the purification process. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads. If the KingFisher Magnetic Beads are inefficiently collected from the elution step, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results. Alternatively, centrifuge the eluates or place them on a magnet for a few minutes to collect the residual beads at the bottom of the well. Carryover of the KingFisher Magnetic Beads does not affect most downstream processes. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the NA yield, as the viral NA has already been released from the KingFisher Magnetic Beads into the eluate. If the KingFisher magnetic particle processor does not work properly, refer to the relevant user manual of the KingFisher instrument in use.
Inhibition of downstream enzymatic reactions	If the purified NA contains residual salt, use the correct order for the Wash Buffers. Always wash the KingFisher Magnetic Beads with Wash Buffer 1 first and then proceed with Wash Buffer 2.

B

Ordering Information

Table B-1. KingFisher Pure Viral NA Kits

Cat. No.	Product	Package size
98070196	KingFisher Pure Viral NA Kit	96
98070496	KingFisher Pure Viral NA Kit	384

Table B-2. KingFisher Flex consumables

Cat. No.	Product	Package size
97002514	KingFisher Flex 96 tip comb for PCR magnet	80 pcs
97002524	KingFisher Flex 96 tip comb for KF magnet	100 pcs
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table B-3. KingFisher Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip comb for KingFisher Flex 24 deep well plate	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates, and elution strips for 96 samples)	1 box
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs

www.thermoscientific.com

Thermo Fisher Scientific
81 Wyman Street
Waltham, MA 02451

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific